

# PCR protocol

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RBRC 04399

Strain name aquaporin 4 (GFP)-2 (Acc.No. CDB0758K-1)

| Primer | primer_name | primer_seq                 | length |                    |
|--------|-------------|----------------------------|--------|--------------------|
| 1      | E1-WT-S     | CTTTCAAAGGAGTCTGGACTCAGGC  | 25     | Depositor's primer |
| 2      | E1-C-AS     | CCAACCACACTGGGAGGTGTGACC   | 24     | Depositor's primer |
| 3      | Neo         | CATCGCCTTCTATCGCCTTCTTGACG | 26     | Depositor's primer |
|        |             |                            |        |                    |
|        |             |                            |        |                    |
|        |             |                            |        |                    |
|        |             |                            |        |                    |
|        |             |                            |        |                    |
|        |             |                            |        |                    |
|        |             |                            |        |                    |

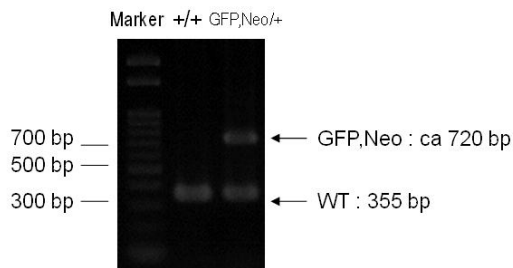
## Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

## Reaction Conditions

|            | Conc             | Run 1 | Run 2 | Run 3 | Run 4 |
|------------|------------------|-------|-------|-------|-------|
| H2O        |                  | 12.3  |       |       |       |
| PCR buffer | 10 X             | 2.0   |       |       |       |
| dNTP       | 2.5 mM           | 1.6   |       |       |       |
| primer 1   | 10 pmol/ $\mu$ l | 1.0   |       |       |       |
| primer 2   | 10 pmol/ $\mu$ l | 1.0   |       |       |       |
| primer 3   | 10 pmol/ $\mu$ l | 1.0   |       |       |       |
| DNA        |                  | 1.0   |       |       |       |
| Taq        | 5U/ $\mu$ l      | 0.1   |       |       |       |
|            |                  |       |       |       |       |
|            |                  |       |       |       |       |
|            |                  |       |       |       |       |
|            |                  |       |       |       |       |
|            |                  |       |       |       |       |
|            |                  |       |       |       |       |
|            |                  |       |       |       |       |
| total      |                  | 20    |       |       |       |

| step | temp (°C) | time (sec) | note                  |
|------|-----------|------------|-----------------------|
| 1    | 94        | 180        |                       |
| 2    | 94        | 30*        |                       |
| 3    | 60        | 30*        |                       |
| 4    | 72        | 60*        | *Repeat for 30 cycles |
| 5    | 72        | 300        |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |



## Marker

Gene Ladder 100 (Wako, Cat. 316-06951)

## Gel

2% agarose in 1 X TAE (100 V, 30 min)

## PCR products

| primer_set |          | product_size | product |
|------------|----------|--------------|---------|
| primer 1   | primer 2 | 355 bp       | WT      |
| primer 2   | primer 3 | ca 720 bp    | GFP,neo |
|            |          |              |         |
|            |          |              |         |
|            |          |              |         |

Comment